Genetic analysis of brown teal (*Anas chlorotis*) from Fiordland

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1. Introduction

The taxonomic relationships and specific status of the New Zealand representatives of Australasian teals have recently been evaluated on the basis of biochemical genetic data (Daugherty et al. 1999). Patterns of genetic divergence supported the recognition of Auckland and Campbell Island populations as full species (*Anas aucklandica* and *A. nesiotis*, respectively).

However, the New Zealand brown teal, *Anas chlorotis*, was represented only by samples taken from birds on Great Barrier Island. Formerly this species occurred throughout the three main islands of New Zealand, as well as on some offshore islands and the Chatham Islands. Now its distribution is limited to Great Barrier Island, the North Island north of Auckland, and Fiordland.

The extreme disjunction of mainland populations of *A. chlorotis* suggests the possibility of significant variation occurring within this species. Genetic diversity within *A. chlorotis* has not previously been assessed. Fiordland populations are of high conservation significance as one of the few surviving remnants of this once widespread species, but their significance could be increased if they were genetically distinct (Molloy & Davis 1992).

This study describes variation in allozyme loci among Great Barrier Island and Fiordland brown teal, Auckland Island teal, Campbell Island teal, grey duck and mallard. Results are used to evaluate the taxonomic and conservation status of Fiordland brown teal.

2. Materials and methods

2.1 SAMPLE COLLECTION

Blood samples were taken from the brachial vein of each of 18 individuals representing Australasian teals, grey ducks and mallards (Table 1). Additionally, seven livers were available for analysis from Fiordland mallards.

Blood samples were placed on ice immediately and centrifuged within several hours. Separated fractions (erythrocytes and plasma) were stored in liquid nitrogen in the field and then transferred to a freezer at -80 ° C for long-term storage.

2.2 ELECTROPHORETIC TECHNIQUES

Tissues were subjected to starch gel electrophoresis according to the techniques of Allendorf et al. (1977). Erythrocytes were mixed with an equal vol-

ume of distilled water prior to electrophoresis, while plasma was used at full strength. A sample of the supernatant was transferred by a filter paper wick to a 12.5% horizontal starch gel (Sigma starch, catalogue no. S4501). Direct current was applied to the gel for 3-4 hours.

The combinations of the. three gel/electrode buffer systems and the 12 different protein (mainly enzyme) stains reported by Daugherty et al. (1999) were examined for electrophoretic activity using the blood samples collected for this study (Table 1). Several additional enzymes were also investigated in the hope of obtaining information from more loci. Liver samples of Fiordland mallards were examined only for variation at the Mpi-1 locus.

2.3 ALLOZYME NOMENCLATURE

We followed the nomenclature of Daugherty et al. (1999) in labelling enzymes, genetic loci and alleles; e.g. *Ak-1*(b) refers to the b allele at the most cathodal locus encoding the enzyme adenylate kinase. Genotypes were assigned to individuals as in that study.

3. Results

Twelve presumed genetic loci were resolved consistently. Ten of these *(Gp-2, GP-3, Gp 5, Gpi-1, Hb-1 Ldh-1, Ldh-2, Mdh-2, Pep-2* and *Sod-1)* showed no variation. One of these, *Ldh-1,* was not reported by Daugherty et al. (1999). Three of the loci reported by Daugherty et al. (1999) (*Ak-1, Mdh-1* and *Pgd-1*) could not be resolved in this study. The two variable loci possessed either two or three alleles (Table 2).

Among the Australasian teals, patterns of divergence detected by Daugherty et al. (1999) were confirmed. At *Est-1* , Campbell Island brown teal exhibited allele b, in contrast to North Island brown teal and Auckland Island brown teal (allele a). Grey duck and mallards exhibited allele b. At *Mpi-1* , Auckland Island brown teal (allele b) differed from Campbell Island and North Island brown teal (allele a). Mallards exhibited allele a, and grey duck had both alleles a and b.

North Island and Fiordland brown teal were both fixed for Est-1(a), but a surprising result was obtained for *Mpi-1.* North Island brown teal were fixed for allele a, and five of six Fiordland brown teal were also homozygous for the a allele. However, the sixth individual was homozygous for an allele never seen before, c. This result is unusual in that uncommon alleles are expected to be seen more frequently in the heterozygous condition than as homozygotes.

To test the possibility that the *Mpi-1*(c) allele is the result of hybridisation with mallard ducks, we investigated the genotype of seven Fiordland mallards from livers made available to us. All of those were homozygous for *Mpi-1*(a),

giving no evidence to support the possibility of hybridisation, at least with mallards.

4. Discussion

At 11 loci, Fiordland brown teal were fixed for the same allele which is fixed in Great Barrier brown teal. At the remaining locus, *Mpi-1,* a low/moderate frequency allele (c) was identified only in the Fiordland brown teal. This population thus appears to be characterised by a unique allele that distinguishes it from northern brown teal.

There is no obvious single explanation for the Fiordland brown teal with the unique *Mpi-1* genotype. One possibility is that the allele represents variation unique to the Fiordland population. In this case, it would provide evidence of significant genetic divergence from the Great Barrier population, as no *Mpi-1* (c) alleles were identified in 58 individuals genotyped by Daugherty et al. (1999). This hypothesis could be investigated by examination of allozyme genotypes in further samples of Fiordland brown teal. Examination of any remaining birds from the mainland North Island should also be a priority.

On the other hand, the bird could be the progeny of hybrids between Fiordland brown teal and other species found in Fiordland, especially mallards. This hypothesis could be investigated by examination of allozyme genotypes in further samples of Fiordland mallards from the location where the brown teal occur. Examination of further Fiordland brown teal could also reveal the occurrence of other individuals with the c allele, confirming it as a characteristic of this population.

5. Taxonomic implications

Generally, birds have relatively low levels of genetic differentiation associated with specific and infra-specific boundaries. Thus, even the low level of differentiation we have found between Fiordland and Great Barrier Island populations of brown teal could be taxonomically significant. However, in the absence of fixed differences, we do not recommend any change to taxonomic status - that is, we believe the pattern and level of allozyme divergence indicates that the two populations of brown teal are probably conspecific.

6. Management recommendations

Fiordland brown teal already have high conservation status, but their population status in Fiordland is uncertain. If populations are declining, their future is not assured. Ecological investigations are required to determine if numbers are changing, but only genetic investigations can confirm if the Fiordland population is at risk due to hybridisation. We recommend further population genetic studies of Fiordland brown teal using both allozyme and mitochondrial methods to evaluate the threats that hybridisation poses. Additionally, these studies should be able to confirm if the Fiordland population differs significantly from northern populations.

7. References

Daugherty, C.H., M. Williams, JM Hay. 1999. Genetic differentiation, taxonomy, and conservation of Australasian teals *Anas* spp. *Bird Conservation International* 9: 29-42.

Molloy, J.,A. Davis. 1992. *Setting priorities for the conservation of New Zealand's threatened plants and animals.* Department of Conservation, Wellington.

Table 1. Species, localities of origin, and numbers of individuals used in this study

Species	Common Name	Locality	Number of Individuals
Anas chlorotis	brown teal	Fiordland-sourced	6
Anas chlorotis	brown teal	ex. Great Barrier Island	3
Anas aucklandica	Auckland Island teal	in captivity	2
Anas nesiotis	Campbell Island teal	ex Dent Island 1990	2
Anas superciliosa	grey duck	from Wellington and Taranaki	3
Anas platyrhynchos	mallard (blood only)	ex Nga Manu Trust	2
Anas platyrhynchos	mallard (liver only)	Fiordland	7

Table 2. Allozyme frequencies for variable loci identified in blood of six populations (four of Australasian teals, one of mallard and one of grey duck).

Locus (allele)	Fiordland brown teal	North Island brown teal	Auckland Island brown teal	Campbell Island brown teal	grey duck	mallard
Est-1 (a)	1.0	1.0	1.0	-	-	-
Est-1 (b)	-	-	-	1.0	1.0	1.0
Mpi-1 (a)	0.83	1.0	-	1.0	0.75	1.0
Mpi-1 (b)	-	-	1.0	-	0.25	-
Mpi-1 **(c)**	0.17	-	-	-	-	-